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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	BOMBARDELLI *et al.*	Confirmation No.:	1328
Serial No.:	10/019,252	Art Unit:	1626
Filed:	December 28, 2001	Examiner:	Saeed, Kamal A.
For:	TAXANE DERIVATIVES AND PROCESSES FOR THE PREPARATION THEREOF	Attorney Docket No:	007914-0085-999 (CAM 105661-(999084)

DECLARATION OF EZIO BOMBARDELLI UNDER 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Ezio Bombardelli, do declare and state as follows:

1. I am a citizen of Italy residing at Via Val di Sole, 22, Milano, Italy, 20141.

2. I am a co-inventor of the invention described and claimed in the above-identified patent application ("the '252 application").

3. I received a degree in Biological Science from the University of Pavia in 1962. From 1962 to 1989, I was a researcher, and then held the position of Head of the Research Laboratory at the company Inverni della Beffa S.p.A. - Milan. Since 1986, I have been Scientific Director at INDENA S.p.A., Milano. I am the author of more than 80 scientific publications, most of which concern medicinal plant derivatives. I am the inventor or co-inventor listed on more than thirty patents all concerning medicinal plant derivatives. I am a member of the Italian Chemical Society, Fédération Internationale Pharmaceutique (F.I.P.), Gesellschaft für Arzneipflanzenforschung and Phytochemical Society of Europe.

4. I have read and am familiar with the above-captioned '252 application and the January 2, 2004 non-final Office Action. I understand the Examiner has rejected claims 32 and 33 under 35 U.S.C. § 112, first paragraph, alleging that the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

5. The compound of Formula I of the '252 application ("Compound I") and a reference compound, ("paclitaxel"), were comparatively assayed for their cytotoxic activity against different tumor cell lines.

6. The following human carcinoma cell lines were used: MCF-7 breast carcinoma cells ("MCF-7"), drug-resistant MCF-7 cells ("MCF7/R"), drug-resistant A2780 ovarian carcinoma cells ("A2780"), and drug-resistant colon adenocarcinoma HCT-15 cell line ("HCT-15").

7. The inhibitory effects of the tested compounds on cell growth were assessed by use of sulforhodamine B (SRB) (Sigma Chemical Co.), a dye-based assay, which indirectly determines cell number by measuring membrane-associated proteins. 1×10^5 exponentially proliferating cells were seeded on 96-well microtiter plates in complete growth medium and incubated at 37°C for 15-18 hours to allow the cells to attach to the substrate before the compounds (i.e. Compound I and paclitaxel) were added. For each compound tested, five 96-well microtiter plates were screened in parallel. All cell lines were exposed to 10-12 different concentrations of each compound, covering a 5- to 6-log range of concentrations, for 72 hours at 37°C, 5% CO_2. Cells were fixed *in situ* for 1 hour at 4°C with ice-cold 50% trichloroacetic acid. The plates were then washed six times with water, and 150 ml of 0.4% SRB was added to each well. After a 5-minute incubation at room temperature, the plates were rinsed with 0.1% acetic acid and air-dried. Bound SRB was solubilized by adding a 100μL 10mM Tris base (pH 10.5) per well and was allowed to stand at room temperature for 5 minutes. The optical density ("OD") of each well was measured at 570nm. Under these conditions, cell number is proportional to OD.

8. We determined the concentration of each drug that inhibited 50% of cell growth ("IC_{50}") using empirically determined concentrations of drugs between 10pM to 30mM; the IC_{50} was obtained by plotting a concentration-effect curve. The resistant index ("RI"), which is a measure of cellular resistance to a particular chemotherapeutic agent, was calculated by dividing the IC_{50} obtained for a drug-resistant cell line by the IC_{50} of the corresponding drug-sensitive cell line (MCF-7R IC_{50}/MCF7 IC_{50}). The cytotoxic activities of Compound I and paclitaxel are reported in the following Table:

Treatment	**IC_{50} nM**				
	MCF7	**MCF7/R**	**RI** (for MCF-7 breast carcinoma cells)	**A2789**	**HCT-15**
Paclitaxel	2.1	920	438	9	273
Compound I	1.4	14	10	4.5	25

9. The cytotoxic potency of Compound I was compared to that of paclitaxel against a panel of human tumor cell lines in the Table of paragraph 8. The results indicate that Compound I displays a wide spectrum of cytotoxicity and is more potent than paclitaxel in all of the tested cell lines. Furthermore, these results show that Compound I is effective in treating diverse types of cancer cells. Moreover, Compound I proved particularly effective against drug-resistant cell lines, significantly reducing the RI of MCF7/R cells.

10. I declare further that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 23.06.04

Ezio Bombardelli